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Isolation and characterization of microsatellite loci in the North Island brown kiwi, *Apteryx mantelli*

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Abstract

We report the isolation and characterization of eight polymorphic and five monomorphic microsatellites in North Island brown kiwi (NIBK, *Apteryx mantelli*), using two polymerase chain reaction (PCR) techniques employing either short-tandem repeat primers (STR method) or random PCR-based isolation of microsatellite arrays (PIMA method). Microsatellite polymorphism was subsequently determined using 65 individuals. There were two to seven alleles for each polymorphic locus with heterozygozity ranging between 0.04 and 0.86. These primers will be used in future studies to determine the level of extra-pair copulation, dispersal patterns, and genetic diversity within and between wild populations of NIBK.

Keywords: *Apteryx mantelli*, brown kiwi, microsatellites, PIMA method

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The North Island brown kiwi (NIBK) is a nocturnal ratite endemic to New Zealand whose populations are declining due to mammalian predators and human encroachment (McLennan *et al*. 1996; Billing 1998). NIBK appear to form strong pair bonds; however, the extent of extra-pair copulation is unknown (del Hoyo *et al*. 1992; Taborsky & Taborsky 1999). NIBK chicks remain near the nest burrow for several weeks posthatch, but little is known about the subsequent dispersal pattern. Eight polymorphic microsatellites have been isolated to facilitate the study of these and other conservation issues.

Genomic DNA (gDNA) was isolated from the blood of a single captive female at the San Diego Zoo. Approximately 1 μL packed red blood cells was suspended in 8% Chelex 100 resin (Sigma), incubated at 56 °C for 30 min, vortexed 30 s and boiled for 8 min. Following centrifugation at 7000 *g* for 15 min, the supernatant was stored at –20 °C until used in polymerase chain reaction (PCR) (Walsh *et al*. 1991; Jensen *et al*. 2003). Microsatellites were identified by two different approaches, short-tandem repeat primers (STR method) or PCR-based isolation of microsatellite arrays (PIMA method) (Lunt *et al*. 1999).

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For the STR method, PCR was performed in 25 μL reactions with the supplied buffer, 3 mM $MgCl_2$, 200 μM of each dNTP, 0.25 μM of each primer (CA6 or CT6), 0.2 U Taq Pol (Biolase, Bioline) and approximately 50 ng gDNA. PCR cycles were 95 °C for 5 min (95 °C for 30 s, 64 °C for 30 s, 1 °C decrease per cycle, 72 °C for 30 s)$_{12}$ followed by (95 °C for 30 s, 49 °C for 30 s, 72 °C for 30 s)$_{20}$ and 72 °C for 7 min. PCR products were cleaned using Microcon-PCR© spin tubes (Millipore), ligated and transformed using the TOPO TA® cloning system (Invitrogen) according to manufacturer's directions. Colonies were screened for inserts by PCR using M13F/R vector primers. Positive colonies were grown in LB media overnight at 37 °C, prepared for sequencing using SNAP© mini prep kits (Invitrogen), and sequenced on an ABI 3100 (Applied Biosystems). Resulting sequences were aligned to chicken, and primers were designed so that one corresponded to NIBK and the other to chicken sequence adjacent to the microsatellite region. These primers were then used to amplify NIBK gDNA under the following conditions: 95 °C for 5 min (95 °C for 15 s, annealing temperature for 15 s, 72 °C for 15 s)$_{34}$ and 72 °C for 3 min. The PCR products were isolated using Microcon-PCR spin tubes and sequenced to replace the chicken sequence with NIBK-specific primers.

Table 1 Microsatellite loci isolated and characterized in *Apteryx mantelli*

Locus name	GenBank Accession no.	Primer sequences	T_a (°C)	Cloned allele repeat sequence	N_T	A_N	Size range of alleles (bp)	Coromandel			Bay of Plenty		
								N_C	H_E	H_O	N_{BP}	H_E	H_O
KMS18	EF222270	HEX™TGCCTTCTCTGCTTGAG ATCCTCCAAATGCCC	47_{M2}	$(GT)_{16}$	83	7	165–189	23	0.34	0.39	28	0.80	0.86
KMS7R	EF222267	6-FAM™GCTTGTCCCTTTAGATTTAGCGG TGTTTTCCCTCCTACTCAATGCTC	54_{M1}	$(GT)_4(CT)_9$ $CC(CT)_{10}$	165	6	199–209	32	0.58	0.53	29	0.35	0.31
KMS16B	EF222269	CCCCCCACTAAGTCTG 6-FAM™AAGTATTCTTGGTAAACAGG	47_{M2}	$(GT)_8$	65	2	148–150	27	—	—	29	0.24	0.14
KMS14B	EF222268	6-FAM™GCTAACATTCACTTGGCATC TGAATCCCTTGGATACTGAGA	54_{M1}	$(AAAT/C)_N$	163	5	268–349	32	0.46	0.44	24	0.04	0.04
KMS37	EF222272	TTCCAGAGCACACACTTAG HEX™GCATAGAACTCACATTTGC	54_{M1}	$(AC)_7$	72	2	146–148	33	0.28	0.27	30	0.16	0.10
KMS30	EF222271	CTGTCAAAATCATCTTTACCAC HEX™TTTCTCTGAGTTTCCGTCC	54_{M1}	$(GT)_9N_{10}(GT)_4$ $N_6(GT)_6 \ldots (GA)_{20}$	165	6	344–354	30	0.46	0.37	26	0.62	0.62
KMS1	EF222266	AAAGCAGCCAAGTTTTTC HEX™TGAATGGAGTCAAGGAAG	54_{M1}	$(AT)_5GT(AT)_3GT(AT)_3 \ldots$ $(AT)_5GTN_4(AC)_{11}$	69	3	155–159	34	0.09	0.09	27	0.23	0.26
KMS74B	EF222273	HEX™AAACACCCTCCCTACTTCG GTGATTGGCAAAACCTGC	47_{M2}	$(CT)_6GT(TC)_{12}$	52	3	281–285	19	0.10	0.11	25	0.46	0.20

T_a is annealing temperature; subscripts M1 and M2 represent two different multiplex reactions. A_N is the number of alleles observed in N_T individuals from seven populations of North Island brown kiwi. H_E is expected heterozygosity and H_O observed heterozygosity calculated for each locus using genotypes of N_C adult individuals from the Coromandel and N_{BP} individuals from the Bay of Plenty. Note that KMS16B is monomorphic within the Coromandel.

The PIMA method was applied as previously described by Lunt *et al*. (1999) with slight modifications. gDNA was used as a template in a random amplified polymorphic DNA-PCR with eight different random 10′mers (R10 : 5′-TCACGATGCA-3′; R11 : 5′-CTGTTGCTAC-3′; R12 : 5′-TGGTCACTGA-3′; R14 : 5′-GGAAGTAGTC-3′; R15 : 5′-ATTGCGTCCA-3′; R16 : 5′-TGCCGAGCTG-3′; R17 : 5′-GTTTCGCTCC-3′; R18 : 5′-TGCACTGGAG-3′). The PCR was performed in 25 μL reactions under the following conditions: 95 °C for 5 min (95 °C for 45 s, 32 °C for 1 min, 72 °C for 45 s)$_{30}$ and 72 °C for 7 min. The TOPO TA® cloning kit (Invitrogen) was used for ligation and transformation according to manufacturer's directions. Colonies were screened for microsatellite inserts by PCR using M13F/R vector primers and either $(CA)_6$ or $(CT)_6$ primers. Clones exhibiting two bands indicating the presence of a microsatellite were prepared as described above for positive clones and were sequenced. To genotype individuals, approximately 10 ng gDNA was PCR amplified in a total reaction volume of 10 μL. Loci were amplified in either a multiplex reaction that contained 5 μL QIAGEN® Multiplex Master Mix and 0.2 μM of each primer, or in single reactions that contained: 1 U Platinum® *Taq* DNA polymerase (Invitrogen), PCR buffer (200 mM Tris-HCl (pH 8.4), 500 mM KCl), 1.5 mM $MgCl_2$, 200 μM of each dNTP, and 0.2 μM of each primer. PCRs were completed utilizing an Eppendorf Mastercycler Gradient following the conditions: 95 °C for 15 min (94 °C 30 s, annealing temperature (Table 1) for 3 min, 72 °C 1 for min)$_{35}$ and 60 °C for 30 min. PCR products were visualized on an Amersham MegaBACE Capillary DNA Sequencer and alleles were scored using MegaBACE Fragment Profiler version 1.2.

Each microsatellite locus was initially tested for variability using 13 individuals from six populations of NIBK throughout New Zealand. Five loci [KMS71(EF222262), KMS15 (EF222265), KMS17B(EF222261), and KMS74(EF222263)/KMS74A(EF222264)] were found to be monomorphic, whereas eight loci were found to be polymorphic (Table 1). These eight loci were from separate clones and were subsequently genotyped in an additional 35 adult individuals solely from the Coromandel region and 30 individuals from the Bay of Plenty. Heterozygosities, adherence to Hardy–Weinberg equilibrium, and linkage disequilibrium were determined using GENEPOP on the Web (Raymond & Rousset 1995). All loci were in Hardy–Weinberg equilibrium in the two focal populations ($\alpha = 0.006$), while there was no linkage disequilibrium between pairs of loci ($\alpha = 0.006$). Highly significant linkage disequilibrium was found, however, between one of our loci, KMS30, and Apt35, a locus previously isolated in NIBK by Shepherd & Lambert (2006).

The STR method yielded many potential microsatellite regions, although the alignment with chicken sequences provided few matches useful for designing primers. Although the PIMA method provided a very low frequency of clones containing microsatellites, it may be preferable because screening is simple and rapid.

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